## AN ANTHOCYANIN-FREE VARIANT OF Darlingtonia californica: Newly Discovered and Already Imperilled

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The discovery

The family Sarraceniaceae contains three genera: *Sarracenia*, *Heliamphora*, and *Darlingtonia*. The genus *Sarracenia* has an unsettled taxonomy, but all authorities agree it contains at least eight species. A number of subspecies, varieties, and forms have also been described. *Heliamphora* has a naturally fragmented range and has responded to this by evolving into several taxa. In contrast, even though *Darlingtonia* grows in scattered montane wetlands throughout southwestern Oregon and northern California, the genus has but a single species, and no significant color variants have ever been discovered.

Until now.

In the spring of 1997 I surveyed a *Darlingtonia* seep in the mountains of north-central California. I had first visited the site in the autumn of 1992 after I had learned of it from Hawkeye Rondeau, a naturalist and intrepid seeker of carnivorous plants. This site is particularly interesting because it is at the extreme southeast edge of the range of *Darlingtonia*. The plants grow in a sloped clearing which is densely hidden on all sides by alders. This clearing measures approximately 20 m  $\times$  45 m, and is habitat for a number of other wetland herbaceous genera including *Carex*, *Drosera*, *Mimulus*, *Juncus*, *Orobanche*, *Platanthera*, *Solidago*, and *Veratrum*. Prominent wetland woody plants include *Alnus incana* subsp. *tenuifolia*, *Leucothoe davisiae*, *Pinus contorta* subsp. *murrayana*, *Rhamnus alnifolia*, and *Vaccinium uliginosum* subsp. *occidentale*. *Sphagnum* does not occur at this location.

Since it was early in the season the plants were in flower. *Darlingtonia* inflorescences are similar in plan to those of *Sarracenia*. Each erect peduncle bears a single pendulous flower that has five large yellow drooping sepals. The five translucent red petals are so closely pressed to each other that together they form a protective chamber around the anthers and gynoecium (see Schnell, 1976, figures 4.2 & 4.3). This chamber may only be entered near the petal tips, apparently ensuring that visiting insects will deposit pollen upon the stigma immediately upon arrival. As my ramblings took me to a much smaller (11 m × 13 m) adjoining clearing of plants, I was greatly thrilled to find anthocyanin-free *Darlingtonia* specimens. In this article I describe some of my observations of what I will refer to as these "variant plants," as well as how you may obtain seed of this variety. The precarious conservation status of these variant plants is also discussed.

Anthocyanin is a pigment found in many plants and is the source of red and pink coloration in plants in the *Sarracenia* family. Recent work by Sheridan (1997) indicates that a single mutation can block the production of this pigment in *Sarracenia*. A number of *Sarracenia* mutants have been reported which lack anthocyanin (this is reviewed in Sheridan's work). On occasion *Sarracenia* plants have been found which have abnormal flower colors but which are not completely anthocyanin-free plants. At first I thought the variant *Darlingtonia* plants were such floral mutants, but inspections of their growth crowns revealed a complete absence of red pigmentation in the leaf shoot apexes and developing pitchers. (A subsequent literature search revealed that the plants had previously been noted by a field researcher who thought the plants were merely yellow-flowered; Elder, 1994.) The variant nature of these plants is clearly genetic and not environmental because of the following reasons.

1) Most of the variant plants were growing in full sun so the absence of anthocyanin is not a response to inadequate light levels. Three variant plants that did grow in shade exhibited the same pigmentation characteristics as their sunlit companions. 2) No intermediate color forms were noted. This is parallels the behavior of the anthocyanin mutation in the genus *Sarracenia*.

3) Variant plants grew interspersed with normal plants, so local factors such as chemicals leaching through the soil could be eliminated. Furthermore, the complete suppression of anthocyanin pigments by environmental effects is unknown in Sarraceniaceae.

As the flowering season progressed I carefully surveyed the site using binoculars (I avoided tromping through the seep because it is so delicate). The variant plants occurred in nine separate loose associations, six of which were in the smaller clearing. A total of 105 variant flowers were observed. In comparison, by measuring the flower number density at various locations (typical values were 11—18 flowers m<sup>-2</sup>) and extrapolating over the area of the two clearings, I estimate that approximately 16,000 normal red flowers were produced this year. Even at this site—its only known occurrence—the variant form represents less than 1% of all the plants present. No doubt a few flowers eluded me, but I probably detected all the major associations of flowering specimens. Surveys of nearby *Darlingtonia* sites revealed no other variant plants. Since *Darlingtonia* plants do not have much red coloration in their pitchers, late-season variant plants do not look much different from normal specimens and any future surveys for variant plants must be conducted during the flowering season.

## Obtaining seed for distribution

Immediately after I discovered the variant plants I contacted the owners of the property and obtained permission to pollinate the plants and collect seed. I selected eleven variant flowers long before they matured and bagged them with 1 mm mesh fabric. The stigmatic surfaces of unbagged plants were usually slightly darker-colored a few weeks after their flowers matured. This discoloration appeared on the bagged plants only after I manually pollinated them. From this I conclude that I successfully excluded pollinators and also that pollinators are present even at this site at the extreme edge of the plant's range. It is still unclear what pollinates *Darlingtonia*, but my measures almost certainly frustrated the efforts of any pollinating agents. Incidentally, nearly every unbagged *Darlingtonia* flower in the clearings contained at least one spider (as has been previously reported, *i.e.* Elder, 1994)—it must be challenging to be a *Darlingtonia* pollinator!

I desired seed of pure anthocyanin-free strains for distribution among scientists and horticulturists so I self-pollinated eight of the bagged flowers. To ensure successful pollen transfer each flower was selfed both 28 May and 1 June. Unfortunately only four of these eight bagged flowers survived to produce seed—shortly after pollination the other flowers died from trauma associated with being handled. Years of experience in *Sarracenia* propagation have shown me that the progeny of such selfings are often not as vigorous as that from cross-pollinated plants. So I pollinated three bagged plants using pollen from two other anthocyanin-free plants (that is, pollen from both pollen donors were applied to each of the three bagged plants). *Darlingtonia* can reproduce vegetatively, both by rhizome division and by stolons. To minimize the chance that the pollen donors were clonally related to the bagged plants, the pollen donors selected were 8 and 11 m from the pollinated plants. Due to handling, only one of these flowers survived to produce seed—*Darlingtonia* peduncles are very brittle, especially when the scientist pollinating them is beset by voracious mosquitoes and gathering lightning storms.

Seed was harvested in September and is available through the ICPS seedbank (see the seedbank listing in this issue). This seed is not regulated by C.I.T.E.S., so it may be bought by any member of the ICPS. To simplify the seed distribution and to increase the chances that the most vigorous seeds are widely distributed, the seed from the pollination trials were mixed together. As a result three different mixes of seed are available.

Mix #1: The results of five manually pollinated variant flowers were combined. Four of the flowers were self pollinated, one was crossed with other variant flowers. These are very likely to be anthocyanin-free plants.

Mix #2: Five unbagged variant flowers were allowed to be pollinated by whatever natural mechanisms are at work in the seeps. If they are selfed then the progeny will be anthocyanin-free. If they were crossed they are very likely to be hybrids with normal flowers. In this case they will probably appear to be normal red-flowered plants.

Mix #3: Seed from nine wild pollinated flowers were collected at the end of the season. The plants are from a unique and wonderful location but will probably produce normal red flowers. Experimenters may wish to use these seeds as controls in studies of the plants from this site.

Seed quartities from this season are limited, but samples from all three mixes are being sent courtesy of the ICPS to other seedbanks around the world. As I write this, cooperative agreements have been arranged with the following organizations (more are being developed): Australian Carnivorous Plant Society (P.O. Box 391, St. Agnes, South Australia 5097), and Gesellschaft für fleischfressende Pflanzen (Frank Gallep, Zweibrückenstr. 31, D-40625 Düsseldorf, Germany). This list of societies is not meant to endorse or snub anyone—it merely represents an eclectic list of societies with which I am familiar. Investigate the seedbanks of your local organizations for possible additional listings.

If you obtain seed, bear in mind you are part of a scientific experiment. I do not know if the plants will breed true. It would be valuable for any growers to report their results to Carnivorous Plant Newsletter.

Conservation concerns and considerations

Field collecting being condoned by the International Carnivorous Plant Society? This is scandalous!

No, not in this case. First, all access to the site was fully allowed by the owners. Second, only seeds were collected, with the exception of a single plant which was used for an herbarium specimen. Third, this situation represents the first find of this plant and is a valid attempt at introducing the plant into cultivation. Fourth, of the 105 variant flowers at the site, only sixteen were manipulated, so the effects from interfering with just one season's flowers are probably insignificant (recall that *Darlingtonia* is a perennial species).

The welfare of the plants is certainly being considered. Indeed, everyone who knows the site has agreed to remain quiet about its location. These measures are justified because plants and fruit of anthocyanin-free *Sarracenia rubra* subsp. *jonesii* have been repeatedly poached from that plant's only known location, in spite of its being a preserve protected and studied by The Nature Conservancy.

The immediate future for this site is precarious. Although it was stewarded well by its owners for many years, when I contacted them they told me they had sold it and the new owners intended to log the property. While the timber value of the seep is minimal, the timber value of the surrounding forest is high. I met with the new owners and their forester. Through nonconfrontational discussions, I was able to educate the owners as to the biological value of the seep. An interesting development occurred several weeks later when the California Department of Forestry (CDF) was reviewing the timber harvest plan for the site. News of this logging operation reached the internet; the resulting deluge of faxes and mail to the CDF was a huge surprise. The CDF consulted with a number of experts (including representatives from the ICPS) and ultimately the property owners volunteered not to log within 100 feet of the seep, so the chances of disturbing the water flow or accidentally felling a tree into the seep were diminished.

At the time of this writing, the site is once again for sale. The Redbud Chapter of the California Native Plant Society (CNPS) is attempting to cobble together enough money to purchase the site. Bound by National Forest on three sides, including the uphill side, this site is excellently situated to be a well-protected preserve. The present owners have agreed to make significant donations to expedite the process, and The Nature Conservancy has contributed a large matching funds grant. Only \$8000 remains to be raised in order to make this 10 acre preserve a reality. That might not seem like much, but for these plants it is the difference between protection and another episode of logging. If you wish to donate to this important project, call Carolyn Chainey-Davis (CNPS, 916-273-1581) by April 1998.

While the International Carnivorous Plant Society played a significant role in protecting this site through educating the land owners and advising the CDF, it must take a back seat to the phase of purchasing the site for protection. When we become a non-profit organization it might be possible for us to be more active in this kind of project.



Figure 1: Anthocyanin-free and normal Darlingtonia flowers.

## Directions for future study

If this site survives the next few years of logging and transitions, it will be an interesting laboratory for the genetic study of anthocyanin-free plants. If Sheridan's work is correct and the production of anthocyanin in *Sarracenia* may be modulated by a single mutation, it is plausible that the trait in *Darlingtonia* is similarly recessive. Indeed, no intermediate flowers were observed (i.e. flowers with pink or only partly red flowers) so this seems likely. It would be interesting to investigate the progeny of green  $\times$  red crosses (using Sheridan's red/green nomenclature). The results from red  $\times$  red or red selfings would also be interesting. It might be that many apparently normal red plants are heterozygous and such pollination's work would result in 25% green seedlings and 75% red seedlings.

By climbing 18 m up into a nearby conifer and photographing the seeps, I produced crude overhead maps. These suggest the anthocyanin-free plants occur preferentially (but not exclusively) at the edges of the seep. The reality of these measurements must be investigated using careful statistical models.

Finally, as information regarding this interesting form accumulates it may be appropriate to botanically describe the variant at the *forma* level. If so, it will most certainly not be "*Darlingtonia californica* f. *heterophylla*," so I do not want to see that invalid name thrown around by growers!

## Acknowledgements:

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